CHANGES IN THE PHYSIOLOGICAL RESPONSE BETWEEN LEAVES AND FRUITS DURING A MODERATE WATER STRESS IN TABLE OLIVE TREES

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Abstract

Pit hardening period is the phenological stage when water stress is recommended in regulated deficit irrigation (RDI) in olive trees. In table olive trees, fruit growth is a very important process which could affect the final profit of the yield. RDI scheduling based on water status measurements could improve water management, but accurate threshold values are needed. Previous works in low fruit load conditions suggested -1.8 MPa of midday stem water potential as “first step” of water stress level where no variations of fruit growth have been detected. The aim of this work is to describe the physiological response of table olive trees with a significant yield in a moderate water stress conditions during pit hardening period. Water relations of Control (no water stress) trees and Stressed trees were studied in a mature table olive orchard in Seville (Spain). Control trees were irrigated with 100% of ETc and values around field capacity were measured. Irrigation in Stressed trees was withdrawn during pit hardening period, and they were irrigated as Control in the rest of the experiment. Fruit growth was not affected until the last days of the deficit period, though midday stem water potential and maximum leaf conductance measurements reached minimum values a few days after the beginning of the water stress period. Such responses suggest two phases in the water stress period. At the beginning of the experiment, the physiological response of the trees (osmotic adjustment and trunk dehydration in the present work) compensated the decrease in water potential. In this phase, leaves and fruits are similar water sink in the shoots. During the last days of the drought period, the reduction of the osmotic adjustment and the greater decrease of fruit water potential transform fruits in more strength water sink than leaves. These changes produced a decrease in the fruit growth. The recovery, though it was not complete, increase fruit size as the same level than Control..

Keywords: Regulated deficit irrigation, recovery, stress integral, water potential, water relations.

1. Introduction

Regulated deficit irrigation (RDI) in olive trees is scheduling with a water deficit period during pit hardening (Goldhamer, 1999). This phenological stage is a dynamic period which can change in length with the water status of the tree (Hammami et al., 2013). In addition, in conditions of significant fruit load in the tree, vegetative growth is stopped (Rallo and Suárez, 1989) and water relations are clearly changed in comparison with low fruit load conditions (Martin-Vertedor et al., 2011). Therefore, pit hardening is a complex phenological stage from the point of view of water relations.

Irrigation scheduling in deficit conditions has been changed in recent decades and there are several works that suggest water status measurements as a more efficient tool than the traditional water balance (i.e. Goldhamer and Fereres, 2001). But all the parameters related directly to the plant physiology could be altered by the drought adaptation process. In olive trees, osmotic adjustment has been suggested as one of the first responses of trees to drought conditions (Dichio et al., 2006). Great dehydration capacity has also been reported as a physiological respond to water stress (Fereres, 1984). Midday stem water potential has been considered the best indicator even in low water stress level (Moriana et al., 2010).

Moriana et al (2012) suggested -1.4 MPa as an adequate threshold value midday stem water potential during the pit hardening period in no water stress conditions. Dell'Amico et al. (2012) in table olive trees reported no decrease in fruit volume in low water stress conditions with minimum values around -1.8 MPa. The low fruit load in this latter experiment is likely to have limited the level of water stress. According to the literatures, such values of water potential are too high for deficit irrigation in olive trees. No clear reduction of fruit yield has been reported with values around -3.5 MPa during pit hardening (Moriana et al., 2003; Iniesta et al., 2009), though fruit growth has been reduced with values higher than -3.0 MPa (Moriana et al., 2013).

The aim of this work is to describe the physiological response of table olive trees in moderate water stress conditions during the pit hardening period. This is the first step to establish a more accurate threshold values or indicators of water potential for irrigation scheduling. We hypothesize that a significant fruit load on the tree will control the process and that shoots water relations would tend to allow fruit growth during the water deficit period.

2. Materials and Methods

2.1. Description of the experiment

Experiments were conducted at La Hampa, the experimental farm of the Instituto de Recursos Naturales y Agrobiología (CSIC). This orchard is located at Coria del Río near Seville (Spain) (37º17'N, 6º3'W, 30 m altitude). The sandy loam soil (about 2 m deep) of the experimental site was characterized by a volumetric water content of 0.33 m^3 m^{-3} at saturation, 0.21 m^3m^{-3} at field capacity and 0.1 m^3m^{-3} at permanent wilting point, and 1.30 (0-10cm) and 1.50 (10-120 cm) g cm^{-3} bulk density. The experiment was performed on 44-year-old table olive trees (*Olea europaea* L cv Manzanillo) during 2012. Tree spacing followed a 7 m x 5 m square pattern. Pest control and fertilization practices were those commonly used by growers and no weeds were allowed to develop within the orchard. Irrigation was carried out during the night by drip using one lateral pipe per tree row and five emitters per plant, delivering 8 L h^{-1} each and spacing 1 m. Irrigation requirements were determined according to daily reference evapotranspiration (ET_o) and a crop factor based on the time of year and the percentage of ground area shaded (40%) by the tree canopy (Kr=0.8). The crop coefficient values (Kc) considered were 0.76 in May, 0.70 in June, 0.63 in July and August, 0.72 in September and 0.77 in October (Fernández et al. 2006).

Trees were irrigated with 100% of crop evapotranspiration (ET_c) in order to obtain non-limiting soil water conditions until the beginning of pit hardening (Phase I). The beginning of the pit hardening was estimated according to Rapoport et al. (2013) around day of the year (DOY) 173. From this date until DOY 233 irrigation was withdrawn in a Stressed treatment (Phase II). All measurements were made in 6 olives irrigated at 100% ET_c throughout the experiment (Control trees) and 6 olives where irrigation was withdrawn (Stressed trees). After DOY 233 trees were irrigated with the same amount of water as Control trees (Recovery). The experiment was stopped at DOY 256 because the harvest had taken place.

2.2 Measurements

Micrometeorological 30 min data, namely air temperature, solar radiation, relative humidity of air and wind speed at 2 m above the soil surface were collected by an automatic weather station located some 40 m from the experimental site. Daily reference evapotranspiration (ET_o) was calculated using the Penman-Monteith equation (Allen et al., 1998).

Soil moisture was measured with a portable FDR sensor (HH2, Delta-T, U.K.) with a calibration obtained in previous works (Fernández and Díaz, unpublished data). This calibration was performed according to the instructions of the sensor and compared the soil moisture measured gravimetrically and the output voltage of the sensor (Equation 1). Equation 1 permits the estimation of the dielectric constant.

$$\theta = 0.4437 * Volt - 0.1697 \ (1); \ (r^2 = 0.76^{***}; n = 59; RMSE = 0.017)$$

Where:

θ: soil moisture measured gravimetrically

Volt: output voltage of the sensor

The measurements were made in four plots per treatment (one access tube per plot). The access tubes for the FDR sensor were placed in the irrigation line around 30 cm from an emitter (Fernández et al., 1991). The data were obtained at 1 m depth with a 10 cm interval.

The drought cycle was characterized by weekly measurements of maximum leaf conductance (g) and midday stem water potential (ψ_{stem}). Abaxial leaf conductance was measured in two full expanded and well illuminated leaves per tree in each treatment with a steady state porometer (LICOR-1600, LICOR, UK) around 10:00 GMT, when maximum values are expected (Xiloyannis et al., 1988). Midday stem water potential in one leaf per tree was measured with a pressure chamber (Model 1000, PMS, USA) around 13:00 GMT. Leaves near the main trunk for ψ_{stem} measurements were covered with aluminium foil two hours before measuring. After the leaf excision, two small cuts parallel to the main nerve were done. These cuts permitted more length in the leaf base to insert in the pressure chamber and increase the grip with the rubber.

In order to describe the cumulative effect of the water deficit, the water stress integral was calculated from the Ψ_{stem} data (Myers, 1988) during the period of water stress (equation 2). In this publication, the integral is estimated with the sum of the surfaces calculated as the average Ψ of two consecutive dates multiplied by the number of days between this dates (Equation 2). Myers (1988) referred the surface to the maximum Ψ measured during the experiment (Equation 2, “c”). Moriana et al (2012) suggested -1.4 MPa of midday stem water potential as reference value in olive trees during pit hardening period. Stress integral was calculated with both reference values in order to compare the usefulness of the Moriana et al (2012) reference. All the values higher than the reference were considered as equal to this. . The expression used was:

$$S\varphi = |\sum(\Psi - c) * n| \qquad (2)$$

where: $S\varphi$ is the stress integral

Ψ is the average midday stem water potential for any interval

c is the maximum value of midday stem water potential in the experiment (traditional use) or the value -1.4 MPa.

n is the number of the days in the interval

The water relations of the leaves and fruits were measured around the time of maximum leaf conductance. Two fully expanded and shaded leaves per tree were randomly cut. Leaf water potential (ψ_{leaf}) was measured with the pressure chamber (Model 1000, PMS, USA) in one of them. This leaf was then covered with aluminium foil and immediately frozen in liquid nitrogen and stored at -80^{o}C. This sample was used to measure actual osmotic potential (ψ_{π}). The second leaf was put in a test tube with distilled water, in which only the petiole was in contact with the water. The test tube was covered with aluminium foil and put into a portable freezer until arrival at the laboratory. Then the test tubes were kept in the dark for 24 hours at 6-8 ^{o}C and then frozen in liquid nitrogen and stored at -80ºC. This sample was used to measure leaf saturated osmotic potential. Fruit water potential was measured with the pressure chamber (Model 1000, PMS, USA) in one fruit per tree in the same shoot where leaf water potential was measured. The fruit was then covered with aluminum foil and immediately frozen in liquid nitrogen and stored at -80^{o}C. This sample was used to measure actual fruit osmotic potential. All frozen tissues (leaf and fruit) were equilibrated at 20ºC for 15 min before determination of osmotic potentials. In the leaf samples, the central nerve was separated from the rest of tissue. Then the tissue was used for the determination of osmotic potential. The osmotic potential was measured after thawing the samples and expressing the sap, using a vapour pressure osmometer (Wescor 5600, Logan, USA). Apoplastic dilution was not considered in any of the osmotic potential measurements. According to Dichio et al (2003), the amount of apoplastic water is very low (lower than 15%) and similar until water stress values of -3.3 MPa of predawn leaf water potential.

Values of turgor pressure (Ψ_p) were calculated as:

$$\Psi_p = \Psi - \Psi_\pi \quad (3)$$

Where:

Ψ_p is the turgor pressure

Ψ is the water potential

Ψ_π is the osmotic potential

Trunk diameter fluctuations were measured throughout the experimental periods, using a set of linear variable displacement transducers (LVDT) (model DF±2.5 mm, accuracy ±10 μm, Solartron Metrology, Bognor Regis, UK) attached to the main trunk, with a special bracket made of Invar, an alloy of Ni and Fe with a thermal expansion coefficient close to zero (Katerji et al., 1994). Measurements were taken every 10 s and the datalogger (model CR10X with AM 416 multiplexer, Campbell Sci. Ltd., Logan, USA) was programmed to report 15 min means. Maximum diameter was measured at the beginning of the day. Trunk growth rate (TGR) in day “n” was calculated as the difference between the maximum daily diameter of day “n+1” minus those of day “n” (Cuevas et al., 2010). TGR is the slope of the figure of maximum diameter. Both parameters, Maximum diameter and TGR, was used as descriptors of the water relations.

The fruit volume was estimated periodically throughout the experiment from a survey of ten fruits per tree (60 fruits per treatment). Two measurements were made for each fruit: the longitudinal dimension and the transversal (at the equatorial point) dimension. At the beginning of the pit hardening period six shoots with fruits per tree were selected randomly. For each shoot the number of fruits were measured at the beginning, the end and in the middle of the period of water stress.

The orchard was divided in two blocks following the slope, then trees inside each block was at the same height in the orchard. Six trees per block (three Control and three Stressed were measured). This design was imposed for the small experimental surface available. Two blocks and two treatments provide no enough repetition to make an ANOVA. Therefore, data of the present work are presented only with the average and standard error because it is no possible to do any significant test. The present work only describe the physiological processes which occur during the water stress but cannot conclude definitively the level of the selected indicators.

3. Results

Climatic conditions during the experiment were typical of the Mediterranean summer, high air temperature and very scarce rainfall, only one day at the end of the drought period (Fig. 1). Reference evapotranspiration (ET_o) was almost constant during the experiment, with a slight decrease from around day of the year (DOY) 200. Annual maximum values of temperature and ET_o were measured during the deficit period of the experiment.

Trees presented clear differences in water relations during the period of water stress (Fig. 2). The soil moisture at 1 m profile was clearly reduced during the stress period (Fig. 2a). Soil moisture in Control trees was around field capacity, while in Stressed trees it decreased until reaching minimum values around DOY 220. Midday stem water potential (ψ_{stem}) and maximum leaf conductance (g_{max}) were lower at stressed trees a few days after the deficit treatment started. ψ_{stem} reached the minimum value around DOY 200, while g_{max} was almost constant, and lower than Control, from DOY 186.

Water relations around the moment of maximum leaf conductance showed differences between leaf and fruit. Leaf water potential (Ψ_{leaf}) showed lower values mainly in the middle of the water stress period (from DOY 207 until 220), though values on the Stress treatment tended to be lower throughout the water stress period (Fig. 3a). On the other hand, lower fruit water potential (FWP) in Stressed trees than in Control were measured from the beginning of the stress period (Fig 3b). The recovery was almost complete at harvest in both parameters though slightly lower values were found in fruit water potential in the last measurement. The difference between leaf and fruit water potential (Ψ_{leaf}-Ψ_{fruit}) showed a change in the pattern during the period DOY 214-233 (Fig. 3c). In Control trees, Ψ_{leaf}-Ψ_{fruit} was around 0 during all the experiment, while in Stressed trees a great increase was measured from DOY 214. Such increase was due to a lower water potential in fruit than in leaves. During the recovery period Ψ_{leaf}-Ψ_{fruit} in Stressed trees decreased and was again similar to Control values.

Leaf osmotic potential in Stressed trees tended to produce lower values than Control at the beginning of the stress period, from DOY 193 to 214 (Fig. 4a). Nevertheless, fruit osmotic potential showed clear differences (Fig. 4b) and the maximum differences were measured at the end of the deficit period. Leaf saturated osmotic potential was slightly lower in Stressed than in Control until DOY 220 (Fig. 4c). From the end of the treatment until harvest, the opposite occurred and Stressed leaf saturated osmotic potential values were slightly higher than Control.

Leaf turgor pressure was affected from DOY 214, before that date values of both treatments were almost equal (Fig. 5a). No clear differences were found during the recovery period, though Stressed values tended to be slightly lower. The variations in fruit turgor pressure were less clear, only from DOY 207 a slight trend of lower values in Stressed trees was measured (Fig. 5b) and at DOY 248, in the recovery period, these differences in the values of fruit turgor pressure in Stressed trees were the greatest.

Maximum diameter before the period of stress was very similar between both treatments. Before pit hardening average trunk growth rate (TGR) tended to produce lower values in Stressed than in Control trees (Table 1 and Fig. 6). In the period of deficit, the diameter decrease of Stressed trees was sharp and continuous almost from the beginning of the period (Fig. 6). TGR was lower in Stressed than Control on several days (Fig. 6) and the average of the period was clearly lower (Table 1). Within the period of water deficit, there were changes in the TGR in both groups of trees though they were greater in Stressed than in Control trees (Table 1). During the recovery period Stressed trees presented a continuous increase with greater TGR than Control (Fig. 6 and Table 1).Such response of TGR values is common when trees are irrigated with a high amount of water after a period of water stress (i.e. in olives Moriana et al., 2003).

Fruit drop was estimated during the period of water stress. There were no differences between treatments in the number of fruits per shoot (Fig. 7). However, there was a clear trend that suggests a fruit drop during the pit hardening period. The number of fruit per shoot in Control trees was around 3 during all the period, while in Stressed trees the number decreased from 3 to 2.3 fruits per shoot. Such decrease supposes a fruit drop around 23% in the number of fruit per shoot.

Fruit growth was continuous throughout the experiment in both treatments (Fig. 8) and the values measured were almost equal. At the end of the period of stress and in the first few days of the recovery, there was a decrease in the rate of fruit growth (from DOY 220) and, then lower fruit volumes were measured in Stressed than in Control trees. However, at the end of the recovery period, from DOY 241, the fruit volume of both treatments were almost equal again. Such differences in the fruit growth pattern suggested a cumulative effect of water stress because minimum values of stem water potential were measured before (DOY 200, Fig. 2). The integral of stress was calculated for the whole stress period and until DOY 214 (Fig. 9). The original Myers' equation (Myers, 1988; equation 2) showed smaller differences between Control and Stressed trees (Fig. 9a) than the ones which included the constant reference value of -1.4 MPa (Fig. 9b). Control values were lower than Stressed in the both considered periods and in both calculations. Control value during pit hardening was around 25 and 7 MPa*day, depending of the reference use, and Stressed trees was 67 and 42 MPa*day..

4. Discussion

Water relations in Stressed trees were affected few days after the beginning of the water withholding. Midday stem water potential, leaf conductance, soil moisture and maximum diameter showed lower values in Stressed than in Control trees. Therefore, water stress conditions started from around day of the year (DOY) 190 (Figs. 2 and 6). According to the water relations parameters, two separated phases in the period of water deficit could be considered. At the beginning, from DOY 190, dehydration of the trees did not affect nor turgor pressure neither fruit growth (Figs. 5 and 8). Such responses could be related with several adaptation mechanisms. There were lower osmotic water potential in leaf and fruit and a trend to lower saturated osmotic potential in Stressed than in Control (Fig. 4). All these results suggest an osmotic adjustment in leaves and likely in fruits. Osmotic adjustment is one of the early mechanisms of trees in respond to water stress (i.e. in olive trees Dichio et al (2003)). In this phase of the water stress period there was, in addition, a fruit drop. In olive trees, fruits are a very important sink of nutrients and water mainly during pit hardening (Rallo and Suárez, 1989). Then, the decrease in the number of sinks could mitigate the initial effects on the rest of the tree. Another possible drought response was the trunk shrinkage. The trunk dehydration in Stressed trees during this phase was clear and could be related with a mitigation of the water stress. Cermak et al (2007) in Douglas fir considered that the water in the trunk/branch is important in the water balance of the tree, mainly in the first weeks of water deficit periods. All these three processes could have delayed the reduction in turgor pressure. Bradford and Hsiao (1982) reported that expansive growth is the most sensitive process to water stress. In the present work, there was no affection of vegetative growth (data not shown) because during pit hardening fruit competition inhibited shoot growth even in full irrigated conditions. According to literature vegetative growth in olive trees is affected before than water potential (Moriana and Fereres, 2002). Therefore, the present work suggests that the fruit growth could be less sensitive to water stress than vegetative growth in olive trees. Caruso et al (2013) reported in a young olive orchard that the yield differences between full and deficit irrigation in a four seasons experiments was due to a decrease in the tree size since the fruit weight and the efficiency of yield for tree size was similar.

This first phase in the water stress period would finish around DOY 214. At this date, there was a decrease of leaf turgor pressure and an increase of $\Psi_{leaf}-\Psi_{fruit}$ (Figs. 3 and 5). Leaf osmotic adjustment at this phase was also reduced since leaf osmotic potential and leaf saturated osmotic potential was almost equal between the two treatments (Fig. 4). These results suggest that at this phase of the water stress period (from DOY 214), fruits are, at the shoot level, the main sink of water. According to Nobel and de la Barrera (2000) positive values of $\Psi_{leaf}-\Psi_{fruit}$ indicated that water entered in the fruit via xylem. In other species, water stress reduced the $\Psi_{leaf}-\Psi_{fruit}$ instead to increase (strawberry, Pomper and Breen, 1997; vines, Greenspan et al., 1996), even in olive trees (Dell'Amico et al 2012). The disagreement with literature results could be related with more strength of the sink in olive than in other fruit trees or with the fruit load, greater in the present work than in the ones of Dell'Amico et al (2012). The change in the hierarchy of fruit within the shoot was accompanied with a reduction of the fruit growth. Then the fruits in no or moderate water stress (as the first phase of the present work) competes with vegetative growth and inhibited it (Rallo and Suárez, 1989). But, according to the present work, fruit at low level of water stress is not a priority water sink, since $\Psi_{leaf}-\Psi_{fruit}$ was changed around 0 (Fig. 3). Similar conclusion was obtained in olive with Dell'Amico et al (2012) at low water stress and low fruit load. When the severity and duration of water stress increases, fruit was a more strength water sink than leaf. The trunk during both phases of the water stress period was a water source for the rest of the tree.

The recovery of trees was completed according to leaf conductance, midday stem water potential and soil moisture (Fig. 2). Although fruit osmotic and fruit water potentials were still below Control at the end of the experiment, the increase in fruit water potential was enough to recover the same fruit size (Figs. 3, 4 and 8). According to this data during recovery the level of water stress in the fruit would be low and similar to the first phase describe above. In such conditions, leaves and fruits are, again, similar water sink as indicated the decrease of Ψ_{leaf}-Ψ_{fruit} (Fig. 3). On the contrary, trunk showed a great increase during all the recovery period, with a TGR greater in Stressed than in Control trees (Fig. 6). This increase during all the recovery period could indicate that trunk is the last priority during rehydration. Sharply increases of TGR during rehydration are commonly reported in olive (i.e. Moriana et al 2003) and others fruit trees (i.e. apples, Swaef et al 2009).

The level of water stress according to midday stem water potential and maximum leaf conductance was almost similar from DOY 200 when the measured values were around the minimum (Fig. 2). The decrease in the number of fruits per shoot (Fig. 7) and the fruit growth (Fig. 8) suggests an additional effect of the duration of the water stress after this date. However, statistical limitations do not allow confirm the accumulative effect of water stress. Bradford and Hsiao (1982) suggested that the result of a water stress is a function of the level, the duration and the moment when it occurred. Myers (1988) in *Pinus radiata* reported than 90% of growth variations were explained for the stress integral (S_Ψ).

However, cumulative effect of water stress is usually not considered in irrigation scheduling likely because there is not a reference to compare. The suggested change in the present work that used the same midday stem water potential as a reference in the Myers's equation supposes a clearer differentiations between Control and Stressed trees than using the traditional parameters (Fig. 9). In addition, the S_{Ψ} values obtained using the value -1.4 MPa could be more comparable between different works.

5. Conclusions

Moderate water stress conditions produced a progressive physiological response in olive trees that divided the water stress period in two phases. At the beginning (from DOY 190 to 214), a group of physiological processes (fruit and leaf osmotic adjustment, fruit drop and trunk shrinkage) delayed the effect of drought in the turgor pressure and fruit growth. At this level of water stress (in some days around -2.5 MPa) fruits and leaves had the same strength as water sink. In the second phase of the period of water stress (from DOY 214 to 232), the reduction of leaf osmotic adjustment likely increased of Ψ_{leaf}-Ψ_{fruit} and fruits received the water mainly via xylem. Such changes produced a reduction in the fruit and leaves turgor pressure and a decrease in the fruit growth. The recovery, although it was not completed, permitted an increase in the fruit size until values similar to Control.

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Table 1. Average of trunk growth rate (TGR) during the three period of the experiment in Control and Stressed trees. The experiment was divided in three phases, which are equal to the ones presented in the rest of figures. Phase I: from the beginning of the experiment until pit hardening (DOY 173). Phase II: from the beginning of pit hardening until recovery (DOY 232). Recovery: which finished at harvest.

	Control (µm día^{-1})	Stressed (µm día^{-1})
Phase I	-0.9±3.2	-5.4±3.3
Phase II	3.2±2.6	-20.8±3.1
173-192	-1.0±3.3	-16.2±3.3
193-201	-11.0±5.6	-41.9±6.1
202-209	23.9±6.1	6.8±5.1
210-224	-10.2±6.3	-35.0±6.7
225-232	25.7±7.9	-9.8±16.0
Recovery	4.5±3.8	22.2±7.4

Figure Captions

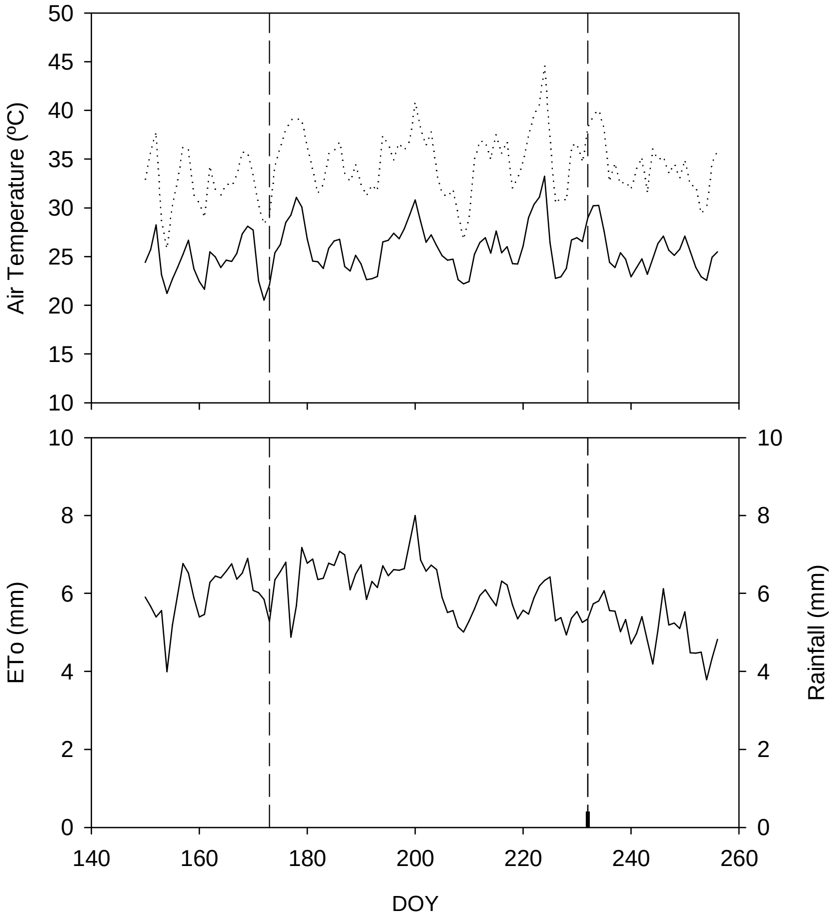

Fig. 1. Climatological data during the experiment. (a) Maximum (dot line) and medium (solid line) temperature. (b) Reference evapotranspiration (ETo, solid line) and rainfall (bar). There was only a one rainfall event at date 233. Vertical lines indicate the period of water deficit.

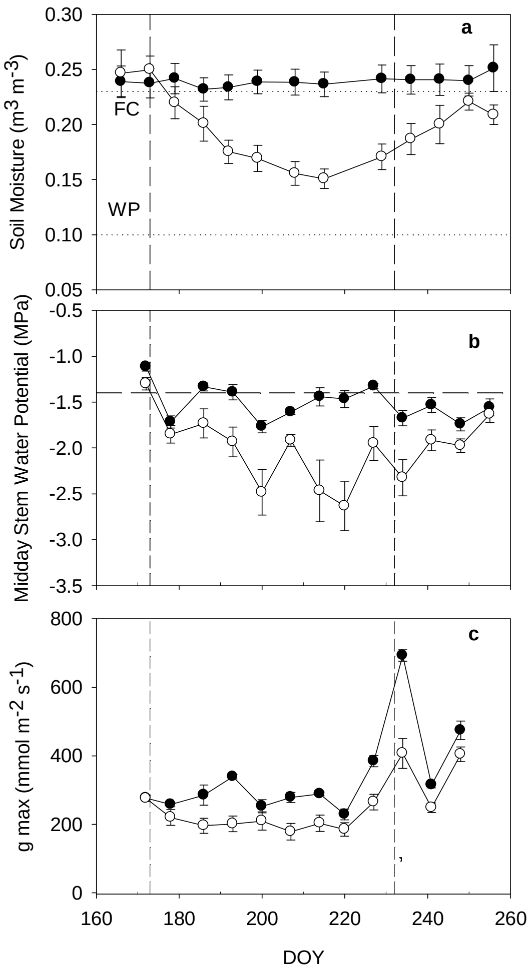

Fig. 2. Pattern of the soil moisture (a), midday stem water potential (b) and maximum leaf conductance (gmax), c) in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 3 (a), 6 (b) and 12 (c) data respectively. Vertical bars represent the standard error. Vertical lines indicate the period of water deficit. The horizontal line in midday stem water potential graph shows the level used as a reference in stress integral (-1.4 MPa).

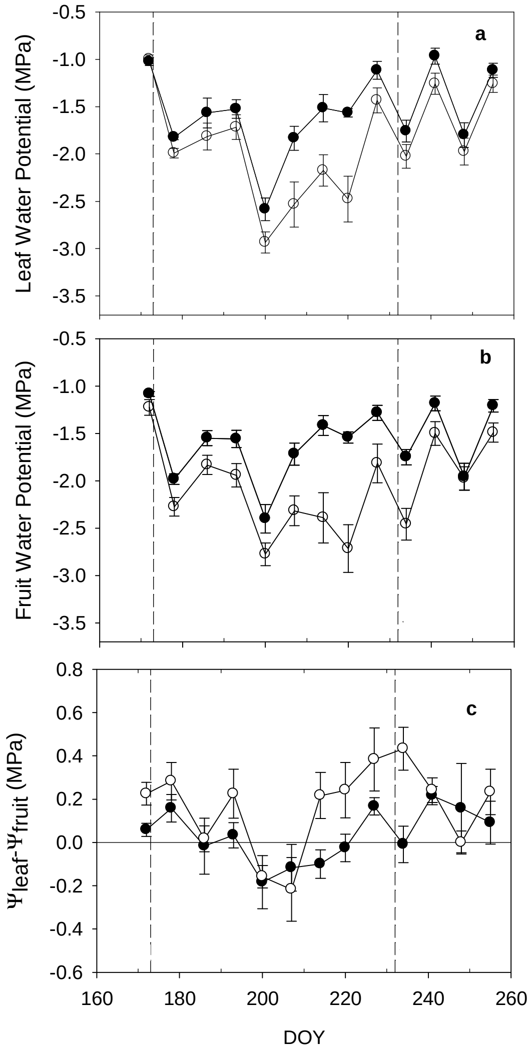

Fig.3. Pattern of leaf water potential (a), fruit water potential (b) and difference between leaf and fruit water potential (ΔΨ, leaf-fruit water potential), c) in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 6 data. Vertical bars represent the standard error. Vertical lines indicate the period of water deficit.

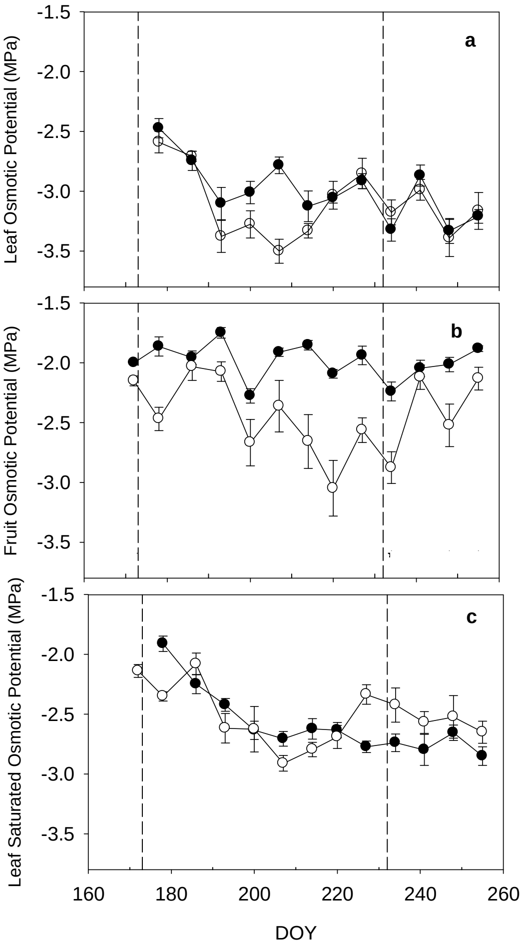

Fig. 4. Pattern of leaf osmotic water potential (a), fruit osmotic water potential (b), leaf saturated osmotic water potential (c) in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 6 data. Vertical bars represent the standard error. Vertical lines indicate the period of water deficit.

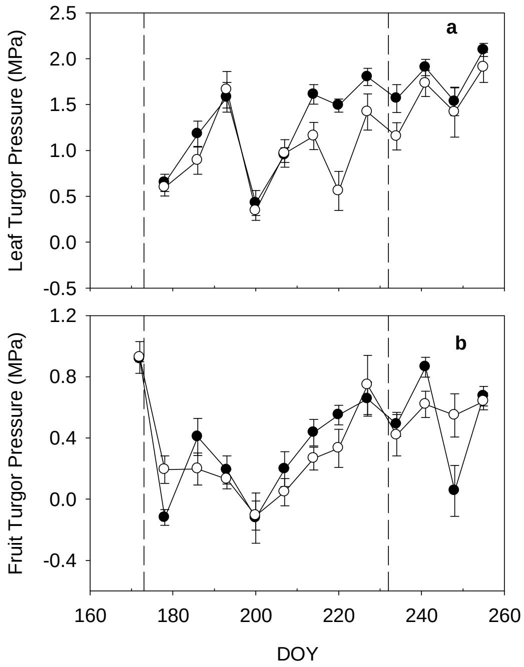

Fig. 5. Pattern of leaf (a) and fruit (b) turgor water potential in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 6 data. Vertical bars represent the standard error. Vertical lines indicate the period of water deficit.

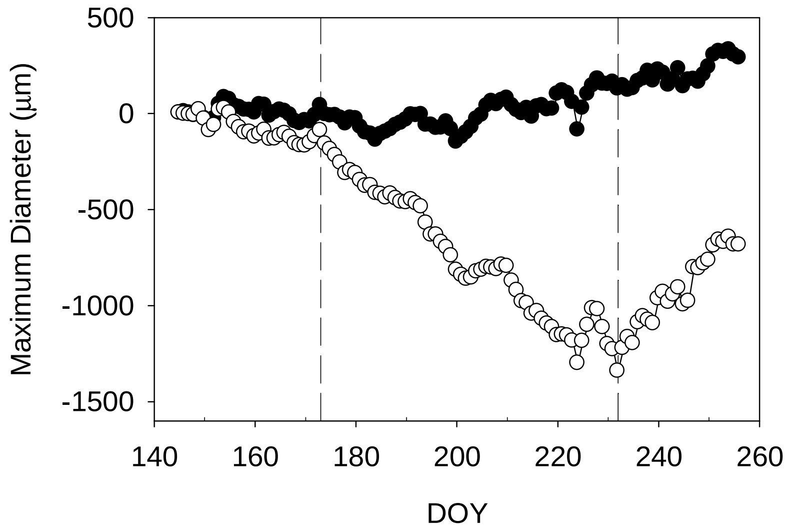

Fig. 6. Pattern of Maximum diameter during the experiment in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 6 data. Vertical bars represent the standard error. Vertical lines indicate the period of water deficit.

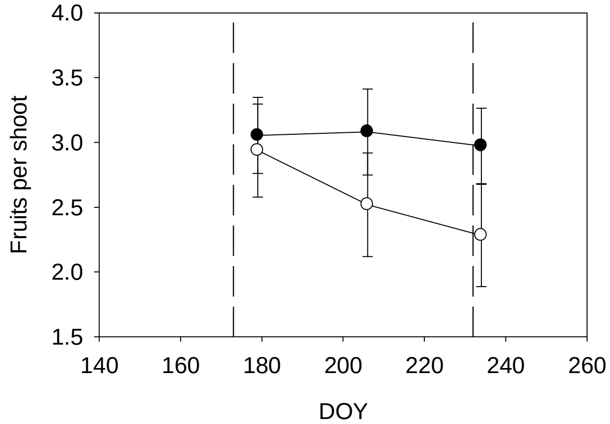

Fig. 7. Pattern of number of fruit per shoot during the experiment in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 36 points. Vertical bars represent standard error.

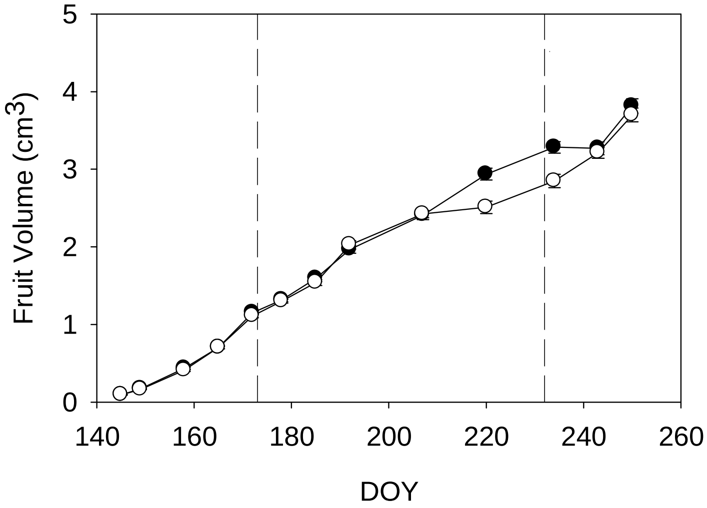

Fig. 8. Pattern of fruit volume during the experiment in Control (solid symbols) and Stressed trees (empty symbols). Each symbol is the average of 60 data. Vertical bars represent the standard error. Vertical lines indicate the period of water deficit.

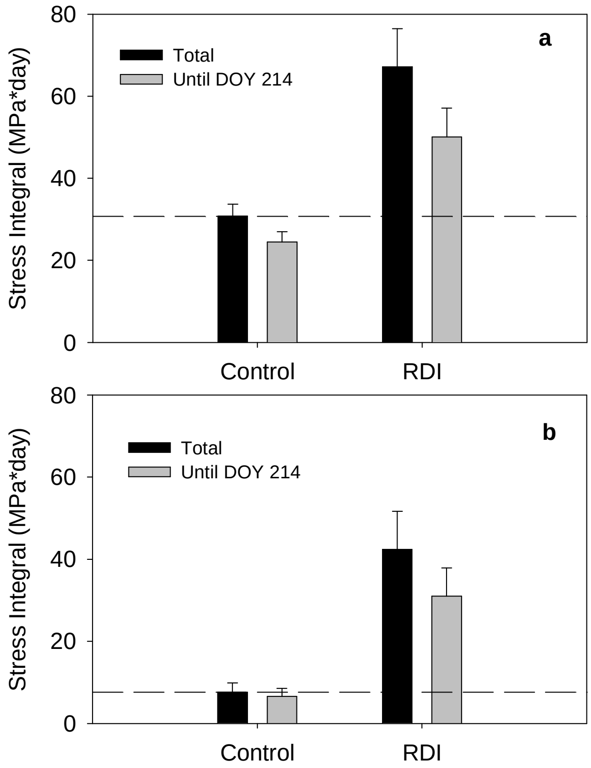

Fig. 9. Stress integral calculated in two different period of the experiment.. (a) Calculation of the stress integral with the maximum value of midday stem water potential in the experiment. (b) Calculation of the stress integral with the reference value of -1.4 MPa Black bars represent the Stress integral in the pit hardening period. Grey bars represent the Stress integral until DOY 214 when fruit volume started to decrease. Each symbol is the average of 6 data. Vertical bars represent the standard error. Horizontal dash line represents the stress integral of Dell’Amico et al (2012) calculates in the two ways suggested.